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(54) Title: NEUROLOGIC AGENTS FOR NASAL ADMINISTRATION TO THE BRAIN

## (57) Abstract

Disclosed is a method for transporting neurologic therapeutic and/or diagnostic neurologic agents to the brain by means of the olfactory neural pathway and a pharmaceutical composition useful in the treatment and diagnosis of brain disorders.

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NEUROLOGIC AGENTS FOR NASAL ADMINISTRATION  
TO THE BRAIN

Field of the Invention

5       The present invention is directed to a method for delivering therapeutic and/or diagnostic neurologic agents to the brain by means of the olfactory neural pathway and a pharmaceutical composition useful in the treatment and diagnosis of brain disorders.

10                   Background of the Invention

Alzheimer's disease is an age-associated neurodegenerative disorder of the brain. The disorder is characterized histopathologically by the formation and accumulation of neurofibrillary tangles (NFT) and neuritic  
15       plaques in the brain. In particular, pathological changes associated with the disease extensively affect neurons in the olfactory bulb and its connected brain structures. Degeneration with loss of neurons has been observed in the hippocampal formation, amygdaloid nuclei, nucleus basalis  
20       of Meynert, locus ceruleus, and the brainstem raphe nuclei, all of which project to the olfactory bulb. These degenerative changes result in the loss of memory and cognitive function. In addition, there is a major loss of cortical and hippocampal choline acetyltransferase  
25       activity and degeneration of basal forebrain cholinergic neurons. The loss of odor detection in Alzheimer's patients has been attributed to necrosis of olfactory epithelium, olfactory bulbs and tracts and the prepyriform cortex.

30       Neurofibrillary tangles (NFT) are believed to result from the abnormal deposition of protein within the neuron structure. Research into the composition and structure of these tangles in isolated NFT-bearing neurons and in partially purified NFT preparations using a  
35       monoclonal antibody, A2B5, suggests that the Alzheimer's neurofibrillary tangles have a glycolipid antigenic marker associated with them. See C. R. Emory, et al., Neurology 37: 768 (1987), and C. R. Emory, et al., Fed. Proceedings 45: 1728 (1986). Previous studies have demonstrated that

A2B5 reacts with polysialated gangliosides such as G<sub>Q1c</sub>. See, N. Kasai and R. K. Yu, Brain Research 277: 155 (1983). Evidence further suggests that the molecular substructures or epitopes characteristic of and specific for Alzheimer's disease, termed "tangletopes" herein, are other glycolipids, sulfolipids, phospholipids, and/or phosphoproteins. It is hypothesized that a structure of the tangletope may involve phosphate and/or sulfate moieties.

At present, there is no treatment for Alzheimer's disease which effectively prevents or retards the progressive neurodegeneration of the brain and the loss of smell and cognitive decline associated with the illness. There is also no definitive method for establishing the diagnosis of Alzheimer's disease antemortem. Neurotrophic and neuritogenic factors, such as nerve growth factor (NGF) and gangliosides, have demonstrated therapeutic effects in animal models and cell cultures which indicate these substances may be of benefit to patients afflicted with Alzheimer's disease. See Frey, W.H., II and T.A. Ala, Progress in Clinical Neuroscience 1:287-303 (1988).

Neurotrophic and neuritogenic factors are agents that affect the survival and differentiation of neurons in the peripheral and central nervous systems. These growth promoting factors are signaling substances that are synthesized in tissues in response to neurons capable of responding to the factor. They bind to receptors on the surface of nerve cells to promote neuron survival and in some cases are incorporated into nerve cell membranes. Studies further indicate that nerve growth factor (NGF), a class of polypeptide signaling substances, may be capable of improving cholinergic functioning which would prevent injury-induced degeneration of basal forebrain cholinergic neurons and improve cognitive functioning. Nerve growth factor (NGF) is known to bind to receptors on axon terminals, and can be internalized and retrogradely transported to the cell body of neurons. See M. Seiler,

Brain Res. 300:33-39 (1984). Other naturally-occurring nerve growth promoting factors include gangliosides, phosphatidylserine (PS), brain-derived neurotrophic factor, fibroblast growth factors, insulin, insulin-like growth factors, ciliary neurotrophic factor, neurotrophin 3, and glia-derived nexin, and other growth factors which are capable of acting within the brain.

Testing the effectiveness of potentially therapeutic agents against brain disease in animal toxicity studies and human trials has been hindered, however, by the inability of existing procedures to readily deliver adequate levels of the agent to affected areas of the brain over an extended period of time.

Some experimental therapeutic agents used in the treatment of Alzheimer's disease, such as GM-1 ganglioside, can be administered to the brain through the bloodstream because of their ability to traverse the blood-brain barrier. However, it is not clear that effective levels of the ganglioside reach the affected areas of the brain.

Other potentially therapeutic agents, such as nerve growth factor (NGF), are unable to cross the blood-brain barrier and must be administered to the brain by other means. One such method of delivery is by an intracerebroventricular pump. Use of such a pump, however, necessitates invasive surgery which can entail a variety of medically-related complications. Furthermore, administration of medication by pump does not facilitate selective delivery of medication solely to those areas of the brain affected by disease. Consequently, healthy areas of the brain may be adversely affected by the neurologic agent while some diseased areas may not receive a high enough level for adequate treatment or testing of a drug.

An effective method of therapeutic intervention is needed to prevent and effectively treat brain diseases such as Alzheimer's disease, Parkinson's disease, brain

tumors, AIDS, nerve damage from cerebrovascular disorders such as stroke, and ordinary aging. Testing the potential of various neurologic agents is an important aspect of developing treatments for neurodegenerative diseases. Since existing methods of testing possible therapeutic agents and treating brain disorders are of limited benefit, a goal of the present invention is to develop a procedure to effectively deliver neurologic agents to the brain. A particular goal of the invention is to develop a method of delivering neurologic substances to the brain to augment the level of activity against brain diseases by naturally-occurring substances. A further goal is to develop a means of selective delivery of a neurologic agent only to areas of the brain which are damaged by a brain disorder. Still another objective is to develop a composition that can cause absorption of the neurologic agent into olfactory neurons and along the olfactory neural pathway to damaged neurons in the brain. Another goal is to provide prophylactic treatment of neurodegenerative diseases and to treat and/or prevent associated loss of smell. Still another goal is to provide a method for the delivery of neurologic diagnostic reagents to the brain in order to improve the diagnosis and evaluation of patients with neurodegenerative diseases and other brain disorders.

#### Summary of the Invention

These and other goals are met by the present invention which is directed to a method to convey therapeutic and/or diagnostic substances to the brain for the treatment and/or diagnosis of neurologic or psychiatric disorders and a pharmaceutical composition capable of delivering a neurologic agent to the brain for use in such a method of treatment and/or diagnosis. More specifically, the method involves intranasal administration of a neurologic agent which may be absorbed into the olfactory system of the brain for the treatment

and/or diagnosis of brain diseases and disorders such as Alzheimer's disease, Parkinson's disease, brain tumors, AIDS, schizophrenia, affective disorders such as depression and mania, anxiety disorders, dependency on  
5     addicting substances, nerve damage from cerebrovascular disorders such as stroke, and brain changes associated with aging. The method also involves administration of a neurologic agent which may be a receptor-active agent, for example, an opiate receptor antagonist, for use in  
10    evaluating the existence and/or level of dependence on addicting substances, for example, cocaine, heroin, and marihuana, or for the treatment of such addictions.

According to the method of the invention, a neurologic substance is administered to the nasal cavity  
15    of a patient affected with Alzheimer's disease or other disease afflicting the brain. The neurologic factor may be applied alone or in combination with other substances. Particular formulations may include the neurologic substance in combination with a pharmaceutically-  
20    acceptable carrier and/or components that may facilitate the transfer of the neurologic agent through the nasal mucosa and/or along the olfactory neural pathway to damaged nerve cells of the brain.

The neurologic agent may be administered  
25    intranasally as a powder, spray, gel, ointment, infusion, injection, or drops. Alternatively, the neurologic agent may be administered as eye drops.

The method of the invention may employ transneuronal anterograde and retrograde transport of the neurologic agent entering through the olfactory system of  
30    the brain. Once the agent is dispensed into the nasal cavity, the agent may transport through the nasal mucosa by means of the peripheral olfactory neurons into the olfactory bulb and interconnected areas of the brain such  
35    as the hippocampal formation, amygdaloid nuclei, nucleus basalis of Meynert, locus ceruleus, and the brainstem raphe nuclei. The agent alone may facilitate this

movement into the brain. Alternatively, the carrier and/or other transfer-promoting factors may assist in the transport of the neurologic agent into and along the olfactory neural pathway.

5           Lipophilic substances in the form of micelles or liposomes (lipid vesicles) may be added to the pharmaceutical composition to enhance absorption of the neurologic agent across the olfactory epithelium. To  
10   augment such absorption, the neurologic agent may be contained within or bound to the surface of the micelles or liposomes. Among those substances that are preferred additives are gangliosides such as GM-1, and phospholipids such as phosphatidylserine (PS), which may be combined  
15   with the neurologic agent either alone or in combination. Substances that are preferred liposome additives are those which provide vesicles bounded by one or more lipid bilayers and are readily soluble in fats, and have an internal cavity filled with a solvent such as water. Suitable liposome additives include those which provide  
20   unilamellar, multilamellar or paucilamellar lipid vesicles. Unilamellar vesicles are preferred.

          Odorants may also be added to the pharmaceutical composition to provide an odoriferous sensation and/or to aid in inhalation of the composition. Preferably, the  
25   odorant agent has an affinity for binding to odorant binding protein (OBP) which may assist in transport of the neurologic agent to olfactory receptor neurons. It is also preferred that the odorant agent has an affinity for associating with lipophilic substances, for examples,  
30   liposomes and micelles added to the composition. Preferred odorants include, for example, terpenoids such as cetrilva and citronellol, aldehydes such as amyl cinnamaldehyde and hexyl cinnamaldehyde, esters such as octyl isovalerate, jasmines such as C1S-jasmine and  
35   jasmal, and musk 89.

          The invention further provides a method for preventing neurodegenerative disorders. Intranasal

administration of nerve growth promoting factors to peripheral nerve cells of the olfactory system, a purported entryway for causative agents of brain diseases, helps protect against disease in these nerve cells and regenerate injured nerve cells thereby forestalling the subsequent spread of disease to susceptible areas of the brain. Although a part of the central nervous system, the neurons of the olfactory epithelium have the unusual ability to proliferate throughout adult life. See Graziadei, P.P.C. and Monti Graziadei, G.A., J. Neurocytol. 8:1-18 (1979).

The invention is also directed to a pharmaceutical composition which may be used in the method of medical treatment and/or prophylaxis. The composition is comprised of a neurologic agent in combination with a pharmaceutical carrier and/or the foregoing optional additives which promote the transfer of the agent within the olfactory system.

The neurologic agent is the active ingredient of the composition. For treatment and/or prophylaxis, it is preferred that the neurologic agent promote nerve cell growth and survival or augment the activity of functioning brain cells such as neurons, glia, and the like. Among those agents that are preferred are neurotrophic and neuritogenic factors that are similar to naturally occurring nerve growth promoting substances or "growth factors." As used herein, the term "growth factor" is synonymous with the term "trophic factor." Among the preferred neurologic agents are trophic factors such as gangliosides, phosphatidylserine (PS), nerve growth factor (NGF), brain-derived neurotrophic factor, fibroblast growth factors, insulin, insulin-like growth factors, ciliary neurotrophic factor, neurotrophin 3, glia-derived nexin, cholinergic enhancing factors, cholinesterase inhibitors, platelet derived growth factors, alpha platelet derived growth factor, transforming growth factor beta, and other growth factors which may be capable of

acting in the brain. GM-1 ganglioside and nerve growth factor (NGF) are particularly preferred. One or several neurologic substances may be combined together.

5       The neurologic agent may further be capable of  
antiviral, antibacterial, antineoplastic, antiparasitic,  
anti-inflammatory, and/or antifungal activity. The agent  
may also be a substance which is capable of acting as a  
neurotransmitter, neuromodulator, nootropic, hormone,  
10       hormone releasing factor, or hormone receptor agonist or  
antagonist. The agent may also be an activator or  
inhibitor of a specific enzyme, an antioxidant, a free  
radical scavenger, a metal chelating agent, or an agent  
which alters the activity of ion channels of brain cell  
15       membranes, for example, nimodipine. The agent may further  
be any substance which is capable of acting as a  
stimulant, sedative, hypnotic, analgesic, anticonvulsant,  
antiemetic, anxiolytic, antidepressant, tranquilizer,  
cognition enhancer, and/or narcotic antagonist or agonist.  
20       Further, the neurologic agent may be any substance found  
to be deficient in conjunction with the brain disorder  
being treated or prevented, for example, nutrients such as  
glucose, ketone bodies, and the like, or metabolic  
precursors such as lecithin (phosphatidylcholine), choline  
or acetyl coenzyme A for producing neurotransmitters for  
25       the treatment of Alzheimer's disease.

30       A preferred embodiment of the therapeutic  
composition is the combination of an effective amount of  
nerve growth factor (NGF) protein with an appropriate  
amount of GM-1 ganglioside in a pharmaceutically-  
acceptable liquid carrier. The GM-1 may function not only  
as an active ingredient of the composition, but may also  
provide lipid vesicles and micelles which may facilitate  
the delivery of the medication by means of the peripheral  
olfactory neural pathway to the brain. GM-1 is thought to  
35       act synergistically with nerve growth factor (NGF) to  
protect neurons and promote nerve regeneration and repair.  
See Gorio et al., Neuroscience 8:417-429 (1983).

For diagnosis of brain diseases or disorders, it is preferred that the neurologic agent is a diagnostic agent, for example, polyclonal or monoclonal antibodies which are capable of detecting substructures or biochemical markers characteristic of the disease or disorder. It has been demonstrated that certain central neurons, especially those neurons of the CNS with axons projecting outside of the blood-brain barrier, are capable of taking up immunoglobulins from the periphery by retrograde axonal transport. See Fabian, R.H. and G. Petroff, Neurology 37:1780-1784 (1987); and Fabian, R.H., Neurology 40:419-422 (1990). Preferably the antibody is monoclonal. Such diagnostic antibodies may be labeled with any labeling agent which may be suitable according to the invention. Suitable labeling agents include, for example, technetium-99m, 123-I, gold or other electron dense particles, positron emitters, and the like. These labels may be detected using appropriate imaging techniques such as single photon emission computed tomography (SPECT), medical resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT), and the like, depending upon the type of label used. Chemical reagents which have an affinity for or are capable of detecting diseased cells or pathologic structures, features, or biochemical markers, including receptors, may also be used as the diagnostic agent. For example, 123-I-quinuclidinyl benzilate (QNB) which binds to muscarinic acetylcholine receptors in the brain and may be imaged with SPECT, and <sup>11</sup>C-nicotine which binds to nicotinic acetylcholine receptors and may be imaged with PET may be used as diagnostic chemical reagents.

A preferred embodiment of a diagnostic composition useful for the diagnosis of Alzheimer's disease comprises an antibody selectively reactive with molecular species ("tangles") associated with Alzheimer's disease, and most preferably with glycolipid, sulfolipid, phospholipid or phosphoprotein antigens. A

preferred composition comprises monoclonal antibody TLE-41, GLE-17, or A2B5. A highly preferred diagnostic composition comprises monoclonal antibody A2B5 labeled with a labeling agent such as technetium-99m, in  
5 combination with a pharmaceutically-acceptable liquid carrier.

The invention is also directed to a method of diagnosing dependency to addicting substances such as caffeine, nicotine, and cocaine, cannabinoids such as  
10 marihuana, opiates such as heroin, and other narcotics. According to this method, a labeled receptor active neurologic agent may be intranasally administered. The receptor active agent is preferably capable of binding with receptors for the particular addicting substance  
15 (i.e., heroin, nicotine) for which addiction is being examined. Labeled receptors may then be detected using an appropriate imaging technique. The number of receptors bound by the labeled agent may also be assessed and/or quantified to evaluate the existence of and/or level of  
20 addiction. For example, an opiate receptor antagonist labeled with technetium-99m may be administered intranasally and imaged to assess and/or measure the level of opiate addiction according to the quantity of labeled receptors.

25 In addition to diagnosing dependency on addicting substances, the invention also provides a method of treatment of such dependency. A therapeutic neurologic agent may be administered intranasally, the neurologic agent being a receptor active agent capable of binding to  
30 a receptor for an addicting substance such as caffeine, nicotine, or heroin, cocaine, opiates and other narcotics. It is preferred that the agent is capable of altering or blocking the receptor so as to interfere with the action of such addictive substances.

35

Detailed Description of the Invention

The method of the present invention administers a neurologic agent to the nasal cavity of a human or other mammal for the testing of potential therapeutic agents against brain disease and for the treatment and/or diagnosis of brain disorders such as Alzheimer's disease, Parkinson's disease, AIDS, brain tumors, schizophrenia, affective disorders such as depression and mania, anxiety disorders, dependency on addicting substances, nerve damage from cerebrovascular disorders such as stroke, or brain changes associated with aging. In particular, the method delivers a neurologic agent to diseased areas of the brain by means of the olfactory neural pathway. The method may employ a pharmaceutical composition capable of transporting the neurologic agent to diseased neurons of the brain.

The method of the invention may achieve delivery of neurologic substances to afflicted areas of the brain through transneuronal retrograde and anterograde transport mechanisms. Delivery of neurologic agents to the brain by that transport system may be achieved in several ways. One technique comprises delivering the neurologic agent alone to the nasal cavity. In this instance, the chemical characteristics of the agent itself facilitate its transport to diseased neurons in the brain. Alternatively, the agent may be combined with other substances that assist in transporting the agent to sites of damaged neurons. It is preferred that auxiliary substances are capable of delivering the agent to peripheral sensory neurons and/or along neural pathways to dysfunctional areas of the brain. It is further preferred that the peripheral nerve cells of the olfactory neural pathway be utilized in order to deliver the neurologic agent to damaged neurons in those regions of the brain that are connected to the olfactory bulb.

The neurologic agent that is administered by the method of the invention may be generally absorbed into the

bloodstream and the neural pathway of the mammal. It is preferred that the agent exhibits minimal effects systemically. For treatment and/or prophylaxis, it is preferred that a large enough quantity of the agent be applied in non-toxic levels in order to provide an effective level of activity within the neural system against the brain disease. It is further preferred that the neurologic agent promote nerve cell growth and survival or augment the activity of functioning cells including enhancing the synthesis of neurotransmitter substances. Among those agents that are preferred are neurotrophic and neuritogenic factors that are similar to or the same as nerve growth promoting substances that are naturally occurring in the nervous system of a mammal. The agent may be administered to the nasal cavity alone or in combination with other neurologic agents. The agent may be combined with a carrier and/or other adjuvants to form a pharmaceutical composition. Trophic factors are among the preferred neurologic agents according to the invention. Preferred trophic factors include gangliosides, nerve growth factor (NGF), phosphatidylserine (PS), brain-derived neurotrophic factor, fibroblast growth factors (FGF) such as basic fibroblast growth factors (bFGF) and heparin-activated acid FGF, insulin, insulin-like growth factors, ciliary neurotrophic factor, neurotrophin 3, glia-derived nexin, and cholinergic enhancing factors such as phosphoethanolamine, L-acetylcarnitine, cholineacetyltransferase development factor (CDF) and thyroid hormone T<sub>3</sub>, cholinesterase inhibitors such as tetrahydroaminoacridine and heptylphyostigmine, platelet derived growth factors, alpha platelet derived growth factor, transforming growth factor beta, and other growth factors that may be capable of acting in the brain. Among those agents that are particularly preferred are GM-1 ganglioside and nerve growth factor (NGF).

The neurologic agent may further be capable of antiviral, antibacterial, antineoplastic, antiparasitic, anti-inflammatory, and/or antifungal activity. The agent may be a substance which is capable of acting as a neurotransmitter, neuromodulator, nootropic, hormone, hormone releasing factor, or hormone receptor agonist or antagonist. The agent may further be any substance which may be capable of acting as a stimulant, sedative, hypnotic, analgesic, anticonvulsant, antiemetic, anxiolytic, antidepressant, tranquilizer, cognition enhancer, and/or narcotic antagonist or agonist. Additionally, the neurologic agent may be a substance found to be deficient for the brain disorder or disease being treated or prevented. For example, potential agents include nutrients such as glucose, ketone bodies and the like, or metabolic precursors such as lecithin (phosphatidylcholine), choline, acetyl coenzyme A, and the like, for producing neurotransmitter substances useful in the treatment of Alzheimer's disease. The agent may also be an activator or inhibitor of a specific enzyme, an antioxidant, a free radical scavenger, a metal chelating agent, or an agent which alters the activity of ion channels of brain cell membranes, for example, nimodipine.

The method of the invention delivers the neurologic agent to the nasal cavity of a mammal. It is preferred that the agent be delivered to the olfactory area in the upper third of the nasal cavity and particularly to the olfactory neuroepithelium in order to promote transport of the agent into the peripheral olfactory neurons rather than the capillaries within the respiratory epithelium. Located high in the vault of the nose, the olfactory area is the only area of the body in which an extension of the central nervous system comes into contact with the environment. Bois, et al., Fundamentals of Otolaryngology, page 184, W.B. Saunders Co., Philadelphia (1989). The invention prefers the transport of neurologic agents to the brain by means of

the nervous system instead of the circulatory system so that potentially therapeutic and/or diagnostic agents that are unable to cross the blood-brain barrier from the bloodstream into the brain may be delivered to damaged  
5 neurons in the brain.

It is preferred that the neurologic agent is capable of at least partially dissolving in the fluids that are secreted by the mucous membrane that surround the cilia of the olfactory receptor cells of the olfactory  
10 epithelium in order to be absorbed into the olfactory neurons. Alternatively, the invention may combine the agent with a carrier and/or other substances that foster dissolution of the agent within nasal secretions. Potential adjuvants include GM-1, phosphatidylserine (PS),  
15 and emulsifiers such as polysorbate 80.

To further facilitate the transport of the neurologic agent into the olfactory system, the method of the present invention may combine the agent with substances that enhance the absorption of the agent  
20 through the olfactory epithelium. It is preferred that the additives promote the absorption of the agent into the peripheral olfactory receptor cells. These peripheral neurons provide a direct connection between the brain and the outside environment due to their role in odor  
25 detection.

Optionally, drug solubilizers may be combined with the agent to improve solubility of the neurologic agent and/or help prevent disruption of nasal membranes which may be caused by application of other additive  
30 substances, for example, lipophilic odorants. Preferred drug solubilizers include amorphous mixtures of cyclodextrin derivatives such as hydroxypropylcyclodextrins. See, for example, Pitha, et al., Life Sciences 43:493-502 (1988), the disclosure of  
35 which is incorporated by reference herein.

The olfactory receptor cells are bipolar neurons with swellings covered by hairlike cilia which project

into the nasal cavity. At the other end, axons from these cells collect into aggregates and enter the cranial cavity at the roof of the nose. It is preferred that the neurologic agent is lipophilic in order to promote absorption into the olfactory neurons and through the olfactory epithelium. Among those therapeutic neurologic agents that are lipophilic are gangliosides, for example GM-1, and phospholipids, for example phosphatidylserine (PS). Alternatively, the neurologic agent may be combined with a carrier and/or other substances that enhance the absorption of the agent into the olfactory neurons. Among the supplementary substances that are preferred are lipophilic substances such as gangliosides, for example GM-1, and phospholipids such as phosphatidylserine (PS). Uptake of non-lipophilic neurologic agents such as nerve growth factor (NGF) may be enhanced by the combination with a lipophilic substance. Other lipophilic substances that may enhance delivery of the neurologic agent across the nasal mucosa include bile salts such as sodium deoxycholate, and detergent-like adjuvants including, for example, polysorbate 80 such as Tween™, octoxynol such as Triton™ X-100, and sodium tauro-24,25-dihydrofusidate (STDHF). See Lee, et al., Biopharm., April 1988 issue:30-37 (1988).

In one embodiment of the method of the invention, the neurologic agent may be combined with micelles comprised of lipophilic substances. Such micelles may modify the permeability of the nasal membrane to enhance absorption of the agent. Among the lipophilic micelles that are preferred are gangliosides, particularly GM-1 ganglioside, and phospholipids, particularly phosphatidylserine (PS). Bile salts and their derivatives and detergent-like adjuvants may also be added as micelle substances. The neurologic agent may be combined with one or several types of micelle substances, and may further be contained within the micelles or associate with their surface.

Alternatively, the neurologic agent may be combined with liposomes (lipid vesicles) to enhance absorption of the neurologic agent into the olfactory system. Preferred liposome additives are those substances which provide vesicles which are readily soluble in fats and which are bounded by lipid with an internal cavity containing a liquid such as water. Preferably the neurologic agent is contained or dissolved within the liposome or associated with its surface. Among those liposome substances that are preferred are phospholipids, such as phosphatidylserine (PS), and gangliosides, such as GM-1. For methods to make phospholipid vesicles, see for example, U.S. Patent 4,921,706 to Roberts, et al., and U.S. Patent 4,895,452 to Yiournas, et al. Bile salts and their derivatives and detergent-like adjuvants may also be added as liposome substances.

Once the agent has crossed the nasal epithelium, the invention further provides for transport of the neurologic agent along the olfactory neural pathway. The agent itself may be capable of movement within the olfactory system. In particular, neurotrophic and neuritogenic substances have demonstrated ready incorporation into nerve cell membranes and an affinity for nerve cell receptor sites. Indications are that these substances are naturally synthesized in tissues in response to neural stimulation and that they subsequently bind to receptors on neurons where they act as nerve growth promoting factors.

Alternatively, the neurologic agent may be combined with substances that possess neurotrophic or neurotogenic properties which, in turn, may assist in transporting the agent to sites of nerve cell damage.

Optionally, an odorant agent may be combined with the neurologic agent to provide an odoriferous sensation, and/or to encourage inhalation of the intranasal preparation to enhance delivery of the active neurologic agent to the olfactory neuroepithelium. The odoriferous

sensation provided by the odorant agent may be pleasant, obnoxious, or otherwise malodorous. The odorant receptor neurons are localized to the olfactory epithelium which, in humans, occupies only a few square centimeters in the upper part of the nasal cavity. The cilia of the olfactory neuronal dendrites which contain the receptors are fairly long (about 30-200 um). A 10-30 um layer of mucus envelops the cilia which the odorant agent must penetrate to reach the receptors. See Snyder, et al., J. Biol. Chem 263:13972-13974 (1988). Use of a lipophilic odorant agent having moderate to high affinity for odorant binding protein (OBP) is preferred. OBP has an affinity for small lipophilic molecules found in nasal secretions and may act as a carrier to enhance the transport of a lipophilic odorant substance and active neurologic agent to the olfactory receptor neurons. It is also preferred that an odorant agent is capable of associating with lipophilic additives such as liposomes and micelles within the preparation to further enhance delivery of the neurologic agent by means of OBP to the olfactory neuroepithelium. OBP may also bind directly to lipophilic neurologic agents to enhance transport of the neurologic agent to olfactory neural receptors.

Suitable odorants having a high affinity for OBP include terpenoids such as cetrarva and citronellol, aldehydes such as amyl cinnamaldehyde and hexyl cinnamaldehyde, esters such as octyl isovalerate, jasmines such as ClS-jasmine and jasmal, and musk 89. Other suitable odorant agents include those which may be capable of stimulating odorant-sensitive enzymes such as adenylate cyclase and guanylate cyclase, or which may be capable of modifying ion channels within the olfactory system to enhance absorption of the neurologic agent.

The invention also provides a means for the prevention of brain disorders particularly in cases where the causative factor enters the brain through olfactory neurons. It is preferred that prophylactic treatments be

employed where evidence indicates neuronal degeneration in the olfactory neurons as in the case of Alzheimer's disease and other related brain disorders. Prophylactic treatment of brain disease may involve the direct or indirect application of neurologic therapeutic agents to the olfactory epithelium. Such agents may be absorbed into the peripheral olfactory nerve cells to protect those neurons from damage from neurotoxins and other insults and thereby prevent the spread of a disease-causing agent into other areas of the olfactory neural pathway and treat and/or prevent the loss of smell which may be associated with neurodegenerative diseases and aging. Although part of the central nervous system, the neurons of the olfactory epithelium are capable of proliferating throughout adult life. See Graziadei, P.P.C. and Monti Graziadei, G.A., J. Neurocytol. 8:1-18 (1979). As in the foregoing methods of treatment, prophylactic therapies may apply the neurologic agent alone or in combination with a carrier, other neurologic agents, and/or other substances that may enhance the absorption of the agent into the olfactory neurons. Potential neurologic agents include trophic factors such as gangliosides, nerve growth factor (NGF), phosphatidylserine (PS), brain-derived neurotrophic factor, fibroblast growth factors (FGF) such as basic fibroblast growth factors (bFGF) and heparin-activated acid FGF, insulin, insulin-like growth factors, transforming growth factor beta, ciliary neurotrophic factor, neurotrophin 3, glia-derived nexin, cholinergic enhancing factors such as phosphoethanolamine, CDF, and thyroid hormone T.3, cholinesterase inhibitors such as tetrahydroaminoacridine and heptylphyostigmine, platelet derived growth factors, alpha platelet derived growth factor, transforming growth factor beta, and other growth factors which may be capable of acting within the brain. GM-1 ganglioside and nerve growth factor (NGF) are among those agents that are particularly preferred for prophylactic treatment of brain disorders.

Potential neurologic agents useful in the prevention and/or treatment of brain disease include those agents which may be capable of antiviral, antibacterial, antineoplastic, antiparasitic, anti-inflammatory, and/or antifungal activity, and those agents which may be capable of acting as a neurotransmitter, neuromodulator, nootropic, hormone, hormone releasing factor or hormone receptor agonist or antagonist. Other potential agents include substances which may be capable of acting as a stimulant, sedative, hypnotic, analgesic, anticonvulsant, antiemetic, anxiolytic, antidepressant, tranquilizer, cognition enhancer and/or narcotic antagonist or agonist. The agent may also be an activator or inhibitor of a specific enzyme, an antioxidant, a free radical scavenger, a metal chelating agent, or an agent which may be capable of altering the activity of ion channels of brain cell membranes, for example, nimodipine. The neurologic agent may further be a substance that is found to be deficient for the brain disorder or disease being treated or prevented, for example, nutrients such as glucose, ketone bodies and the like, or metabolic precursors for producing neurotransmitter substances such as lecithin (phosphatidylcholine), choline, and acetyl coenzyme A, which are precursors for neurotransmitters useful in the treatment of Alzheimer's disease.

To deliver the neurologic agent to the olfactory neurons, the agent alone or in combination with other substances as a pharmaceutical composition may be administered to the olfactory area located in the upper third of the nasal cavity. The composition may be dispensed intranasally as a powdered or liquid nasal spray, nose drops, a gel or ointment, through a tube or catheter, by syringe, by packtail, by pledget, or by submucosal infusion.

As an alternative to administering the neurologic agent directly into the nasal passage, the composition may first be administered to the eye as eye drops. Tears

drain through the nasolacrimal ducts into the nasal cavity and become mixed with nasal secretions. Lactoferrin, a substance in tears and the nasal mucosa, has been identified in the plaques and tangles of Alzheimer's disease. See, Osmond, et al., Neurobiology of Aging 11:284 (1990). It is preferred that eye drops containing the neurologic agent are administered to the eye, and the liquid allowed to drain through the nasolacrimal ducts into the nasal cavity and mix with nasal secretions, wherein the agent is delivered to the brain by means of the olfactory neural pathway according to the method of the invention.

The optimal concentration of the active neurologic agent will necessarily depend upon the specific neurologic agent used, the characteristics of the patient and the nature of the disease or condition for which the treatment is to be used. The neurologic agent may be used alone or in combination with other substances as a pharmaceutical composition at such concentrations as 30 $\mu$ M GM-1 ganglioside, 3nM nerve growth factor (NGF), and 300 $\mu$ M phosphatidylserine (PS). These concentrations are intended only as examples and do not exclude the use of other concentrations.

The invention is further directed to a pharmaceutical composition comprising an amount of a neurologic agent which is effective in treating or preventing brain disorders in a mammal, when administered thereto, in combination with a pharmaceutically-acceptable vehicle such as a liquid or powdered carrier and/or various optional adjuvants. The pharmaceutical composition is particularly useful for treating patients with Alzheimer's disease.

The neurologic therapeutic agent of the pharmaceutical composition may be any substance that promotes the survival of nerve cells and other normal brain cells and prevents their further loss. It is preferred that the agent has minimal systemic effects and

augments the activity of naturally occurring nerve growth promoting factors. Preferably, the agent is capable of acting as a nerve growth promoting factor to prevent degeneration of neurons, to induce regrowth of dendrites and axons, and to augment the function of remaining neurons such as synthesizing neurotransmitter substances. Among the neurologic agents that are preferred are trophic factors such as nerve growth factor (NGF), gangliosides, phosphatidylserine (PS), brain-derived neurotrophic factor, fibroblast growth factors (FGF) such as basic fibroblast growth factors (bFGF) and heparin-activated acidic FGF, insulin, insulin-like growth factors, platelet-derived growth factors, ciliary neurotrophic factor, neurotrophin 3, glia-derived nexin, transforming growth factor beta, and cholinergic enhancing factors such as L-acetylcarnitine, phosphoethanolamine, thyroid hormone T3, and cholineacetyltransferase development factor (CDF), cholinesterase inhibitors such as tetrahydroaminoacridine and heptylphysostigmine, alpha platelet derived growth factor, and other growth factors that may be capable of acting within the brain.

The composition may comprise a neurologic agent which may be capable of antiviral, antibacterial, antineoplastic, antiparasitic, anti-inflammatory, and/or antifungal activity. The agent may also be a substance that may be capable of acting as a neurotransmitter, neuromodulator, nootropic, hormone, hormone releasing factor, or hormone receptor agonist or antagonist. The agent may further be any substance which may be capable of acting as a stimulant, sedative, hypnotic, analgesic, anticonvulsant, antiemetic, anxiolytic, antidepressant, tranquilizer, cognition enhancer, narcotic antagonist or agonist including agents such as carbamazepine which may be useful in the treatment of substance abuse. The agent may also be an activator or inhibitor of a specific enzyme, an antioxidant, a free radical scavenger, a metal chelating agent, or an agent which may be capable of

altering the activity of ion channels of brain cell membranes, for example, nimodipine. The agent may also be a substance found to be deficient for the brain disorder or disease being treated or prevented, for example, nutrients such as glucose, ketone bodies and the like, or metabolic precursors such as lecithin (phosphatidylcholine), choline, acetyl coenzyme A and the like, for producing neurotransmitter substances useful in the treatment of Alzheimer's disease and other brain disorders.

The carrier of the composition may be any material which is otherwise pharmaceutically-acceptable and compatible with the active ingredients of the composition. Where the carrier is a liquid, it is preferred that the carrier is within the range of pH 4.5-8.5. Where the carrier is in powdered form, it is preferred that the carrier is also within an acceptable non-toxic pH range.

Among the optional substances that may be combined with the neurologic agent in the pharmaceutical composition are lipophilic substances that may enhance absorption of the agent across the nasal membrane and delivery to the brain by means of the olfactory neural pathway. The neurologic agent may be mixed with a lipophilic adjuvant alone or in combination with a carrier. Among the preferred lipophilic substances are gangliosides such as GM-1 and phospholipids such as phosphatidylserine (PS). One or several lipophilic adjuvants may be combined with the agent. It is preferred that the lipophilic adjuvant be added as micelles or liposomes.

The pharmaceutical composition may also include odorant substances to provide an odoriferous sensation and/or enhance inhalation of the composition. Odorant agents, preferably with an affinity for odorant binding protein (OBP), may also be included to augment the transport of the neurologic agent to olfactory receptor

neurons. Where lipophilic neurologic agents and/or substances such as liposomes and micelles are included in the composition, it is preferred that the odorant agent have an affinity for the lipophilic substance. Among the preferred odorant agents are terpenoids such as cetalva and citronellol, aldehydes such as amyl cinnamaldehyde and hexyl cinnamaldehyde, esters such as octyl isovalerate, and jasmines such as C1S-jasmine and jasmal, and musk 89. The odoriferous sensation provided by the odorant agent may be pleasant, obnoxious or otherwise malodorous.

The pharmaceutical composition may be formulated as a powder, granules, solution, ointment, cream, aerosol, powder, or drops. The solution may be sterile and otherwise suitable for administration by injection or other means. In addition to the neurologic agent, the solution may contain appropriate adjuvants, buffers, preservatives and salts. The powder or granular forms of the pharmaceutical composition may be combined with a solution and with diluting, dispersing and/or surface active agents. Solutions such as nose drops or eye drops may contain antioxidants, buffers, and the like.

A preferred embodiment of the pharmaceutical composition of the invention is a micellar and/or liposomal suspension of GM-1 ganglioside with an effective amount of nerve growth factor (NGF) combined with appropriate amounts of an odorant such as cetalva, a stabilizer such as microcrystalline cellulose, a suspending agent such as carboxymethyl cellulose or hydroxypropyl methylcellulose, an emulsifier such as polysorbate 80, a preservative such as benzalkonium chloride, an antimicrobial such as phenylethyl alcohol, and a thickener such as dextrose.

The present invention for a method of administering neurologic agents useful in the treatment of brain disorders such as Alzheimer's disease presents several advantages over currently available methods.

The method of the present invention prefers the olfactory neural pathway rather than the bloodstream to deliver agents useful for the treatment of brain disorders such as Alzheimer's disease directly to the brain. Use of the olfactory system to transport a neurologic agent to the brain bypasses the blood-brain barrier so that medications like nerve growth factor (NGF), a protein that cannot normally cross that barrier, can be delivered directly to the brain. Although the agent that is administered may be absorbed into the bloodstream as well as the olfactory neural pathway, the agent provides minimal effects systemically. In addition, the invention provides for delivery of a more concentrated level of the agent to neural cells since the agent does not become diluted in fluids present in the bloodstream. As such, the invention provides an improved method of testing potential therapeutic agents against brain disease and of treating neurodegenerative disorders.

The method provides an advantage by virtue of the intranasal administration of the medication. The olfactory system provides a direct connection between the outside environment and the brain thus providing quick and ready delivery of neurologic agents for treatment of neurologic disorders. Moreover, the means of applying a pharmaceutical composition intranasally can be in a variety of forms such as a powder, spray or nose drops which obviates intravenous or intramuscular injections and simplifies the administration of therapeutic medications. As such, the method of the present invention is an improvement over present methods of direct administration of neurologic therapeutic agents, such as the intracerebroventricular pump.

The application of a neurologic therapeutic agent to the nasal epithelium also helps prevent the spread of certain brain disorders by directly treating peripheral olfactory neurons that are injured by neurotoxins and other insults. Prophylactic treatment of these outlying

nerve cells helps preclude the entrance of disease-causing agents into the brain. This method of treatment is particularly beneficial in cases of Alzheimer's disease where an environmental factor, suspected of being one of the causative agents of the disease, is thought to enter the brain through the olfactory pathway. Application of a neurologic therapeutic agent to the olfactory sensory neurons also in part treats and/or prevents the loss of smell which may be associated with neurodegenerative diseases and ordinary aging. Neurons of the olfactory epithelium are capable of proliferation throughout the adult life. See Graziadei, P.P.C. and Monti Graziadei, G.A., J. Neurocytol. 8:1-18 (1979).

Another advantage of the invention is that it provides delivery of neurologic agents solely to those areas of the brain affected by disease while avoiding unwanted treatment of brain regions which are free of the disease. The method of the invention employs a neurologic agent or other substance that has an affinity for neuron receptor sites in order to facilitate delivery of the agent directly to the brain through the olfactory epithelium.

The invention also provides a means for delivering diagnostic neurologic agents to the nasal neuroepithelium, olfactory bulb and other brain structures. The invention is especially useful for the delivery of diagnostic agents, such as antibodies, which do not easily cross the blood-brain barrier. The invention is also advantageous because it delivers the diagnostic agent principally to those areas of the brain affected by disease.

It is preferred that the diagnostic neurologic agent is capable of detecting substructures associated with Alzheimer's disease, Parkinson's disease, AIDS, brain tumors, cerebrovascular disorders, schizophrenia, affective disorders, psychiatric illness, anxiety disorders, aging, dependency on addicting substances, or

other neurologic disorder or disease. Chemical reagents which have an affinity for or may be capable of detecting diseased cells or pathologic structures, features or biochemical markers, including receptors, may be used as diagnostic agents. An example of a diagnostic chemical agent is basic fibroblast growth factor (bFGF) which binds to pathologic structures in the brains of patients with Alzheimer's disease. See T. Kato, et al., Neurobiology of Aging 11:268 (1990). The bFGF may be labeled and imaged with a variety of imaging techniques. An example of a receptor-based diagnostic agent (receptor ligand) is 123-I-quinuclidinyl benzilate (QNB) which is capable of binding to muscarinic acetylcholine receptors in the brain and can be imaged with SPECT. Other examples of diagnostic agents are 11C-(2) deoxyglucose, 18-fluoro-deoxyglucose, <sup>133</sup>Xe and <sup>11</sup>C-nicotine. It is preferred that antibodies that are used as diagnostic neurologic agents are capable of detecting antigens which are characteristic of the brain disease or disorder. The antibody may be polyclonal or monoclonal. Preferably, the antibody is monoclonal.

It is preferred that antibodies used as agents for the diagnosis of Alzheimer's disease are selectively reactive with glycolipid, sulfolipid, phospholipid, or phosphoprotein antigens. More specifically, it is preferred that the antibodies are selectively reactive with "tangletoes" which are a molecular species that is characteristic of, and specific for, the diagnosis of that brain disease. A "tangletoe" in the context of the invention refers to antigenic phosphate- or sulfate-containing lipid and/or protein compounds, epitopes, or haptens, and to antigenic markers such as molecular substructures, that are associated with Alzheimer's disease. Alzheimer's neurofibrillary tangles are known to contain several phosphoproteins including phosphorylated tau. Highly preferred antibodies for use in the diagnosis of Alzheimer's disease are the monoclonal antibodies A2B5,

5 TLE-41, GLE-17, and any monoclonal antibody against a  
mammalian sulfatide such as bovine sulfatide, or against  
protein phosphate epitopes. For a discussion regarding  
A2B5, see U.S. patent application Serial No. 07/398,079  
10 filed August 24, 1989, the disclosure of which is  
incorporated by reference herein. See also Clements, et  
al., Alzheimer Disease and Assoc. Disorders 4:35-42  
(1990). Also highly preferred for use in the diagnosis of  
Alzheimer's disease is any monoclonal antibody against  
15 protein phosphate epitopes, including monoclonal antibody  
A2B5 which reactivity with phosphoproteins was recently  
discovered by Applicant.

The hybridomas producing monoclonal antibodies  
TLE-41 and GLE-17 have been deposited with American Type  
15 Culture Collection (ATCC), 12301 Parklawn Drive,  
Rockville, Maryland, and have been assigned the following  
ATCC accession numbers: the hybridoma for TLE-41 is HB-  
10521, and the hybridoma for GLE-17 is HB-10520. Cultures  
of these deposited hybridomas will be made available to  
20 the public upon the grant of a patent based upon the  
present application. It is to be understood that the  
availability of a deposit does not constitute a license to  
practice the subject invention in derogation of patent  
rights granted by the United States Government.

25 Besides phosphorylated tau, plaques and tangles  
of Alzheimer's disease are also believed to contain  
certain antigens including ubiquitin, microtubule  
associated protein-2 (MAP-2), phosphorylated MAP-5,  
amyloid beta protein, A-68, heparin sulfate proteoglycans,  
30 lactoferrin, and phosphorylated tau. Consequently, any  
antibody which may be capable of activity against those  
antigens would be suitable as a diagnostic agent according  
to the invention. Examples of useful antibodies include  
those which are anti-ubiquitin, anti-MAP-2, anti-amyloid  
35 beta protein, and so forth. Highly preferred is the  
antibody ALZ-50. See, for example, P. Davies, J. Amer.  
Med. Assoc. 263: 2907-2910 (1990).

Various techniques are employed to produce hybridomas capable of secreting monoclonal antibodies that are selectively reactive with circulating tangletopes, e.g., phospholipid, glycolipid, sulfolipid and phosphoprotein epitopes which reflect the tangletope abnormalities characteristic of Alzheimer's disease. Such techniques for antibody preparation are set forth in M. M. Rapport and Y. Huang in Advances in Experimental Medicine and Biology, Vol. 174, R. W. Ledeen, et al., eds., pp. 15-25 (1984), and in G. S. Eisenbarth, et al., Proc. Natl. Acad. Sci. USA 76: 4913 (1979), the disclosures of which are incorporated by reference herein.

Monoclonal antibody A2B5 is a murine IgM produced by a hybridoma that is commercially available from American Type Culture Collection (ATCC No. CRL1520). This antibody is secreted by a cloned hybridoma formed by the fusion of the murine myeloma P3X63 Ag8 with spleen cells derived from a BALB/c mouse immunized against chick embryo retina cells. Monoclonal antibody A2B5 is known to bind to neurofibrillary tangles and senile plaques characteristic of Alzheimer's disease. This antibody exhibits only minimal reactivity with  $G_{Q1b}$  ganglioside, the tetrasialic ganglioside present in normal adult brain gray matter, and has been reported to be primarily reactive with the tetrasialic acid ganglioside  $G_{Q1c}$ . A2B5 has also been demonstrated to be reactive with sulfatide from human or bovine brain and certain base-labile glycolipids. Furthermore, immuno-TLC studies utilizing A2B5 have demonstrated that A2B5 binds to and detects the sulfated glycolipid, sulfatide, from bovine brain. A2B5 also reacts with phosphoproteins such as phosphovitin and casein, and with phospholipids such as dolichol phosphate by ELISA. A2B5 also reacts with a glycolipid, phospholipid or sulfolipid species from extracts of human brain, Alzheimer's neurofibrillary tangles, and Alzheimer's cerebrospinal fluid (CSF), which migrate on the TLC test plates with the same retention time as bovine sulfatide on

TLC. Therefore, any antibody specific to the detection of these lipids or to phosphoproteins present in Alzheimer's neurofibrillary tangles or neuritic plaques may be used in the Alzheimer's disease detection method of the present invention.

Monoclonal antibody GLE-17 and TLE-41 are murine IgM antibodies that react with Alzheimer's neurofibrillary tangles and label Alzheimer's brain neurofibrillary tangles by immunohistochemistry. Monoclonal antibody TLE-41 has demonstrated reactivity with total lipid extract (TLE) of Alzheimer's cerebral cortex, with certain phosphoproteins such as casein by ELISA, and with human and bovine brain sulfatide by ELISA. Monoclonal antibody GLE-17 has demonstrated reactivity with a crude ganglioside fraction of Alzheimer's cerebral cortex. Although the antigens detected by these antibodies are not yet confirmed, the antigens are suspected as being a sulfolipid, glycolipid, phosphoprotein or phospholipid. Both antibodies, being capable of distinguishing Alzheimer's afflicted brain tissue from normal or non-demented brain tissue, may function as diagnostic agents for that disorder.

The detection of the presence of a characterizing tangletope includes either the use of a label directly bound to the diagnostic antibody agent, or the addition of a labeled second antibody which is reactive against the diagnostic antibody agent. The label may be any of a variety of well known and commonly used labels, for example, a radioactive, enzymatic, or fluorescent group. It is preferred that antibodies are labeled with a labeling agent such as technetium-99m, <sup>123</sup>I, gold or other electron dense particles, positron emitters, and the like. The labeled antibody may be detected using various imaging techniques, for example, single photon emission computed tomography (SPECT), medical resonance imaging (MRI), positron emission tomography (PET), computed

tomography (CT), and the like. The method of detection is matched with the type of label used.

5 A preferred composition useful in the diagnosis of Alzheimer's disease comprises monoclonal antibody A2B5 labeled with technetium-99m or other suitable labeling agent, in combination with a pharmaceutically-acceptable liquid carrier.

10 The invention also provides a method to diagnose an individual's dependence on addicting substances such as alcohol and other chemical compounds, especially those characterized by the popular press as drugs. Addicting substances, for example, may include caffeine, nicotine, and cocaine, cannabinoids such as marihuana, opiates such as heroin, and other narcotics. The method is useful for  
15 the delivery of labeled receptor active neurologic agents which are capable of binding to neural receptors for a particular chemical substance (i.e., heroin, nicotine). Labeled receptors may then be detected using an appropriate imaging technique. Further, the number of  
20 receptors bound by the labeled agent, and the extent of binding with respect to time and the concentration of the labeled agent may also be assessed and/or quantified to evaluate the level or extent of addiction. For example, an opiate receptor antagonist labeled with technetium-99m  
25 may be administered intranasally, imaged with SPECT, and the extent of labeled receptor quantified to assess and/or measure the level of opiate addiction. Similarly, <sup>11</sup>C-nicotine may be administered intranasally, imaged with PET, and the binding of nicotine to receptors quantified  
30 to assess nicotine use and addiction and/or habituation in smokers or those using other nicotine-containing products or substances.

35 Examples of receptor active neurologic agents for use in diagnosing dependence on addicting substances include, for example, naloxone, propiram, nalorphine, cyclazocine, methadone, MET-enkephalin, LEU-enkephalin, beta-endorphin, hexamethonium, mecamlamine,

carbamazepine, and QNB. These agents may be labeled with any labeling agent which is suitable according to the invention. Labeling agents which may be used include technetium-99m,  $^{11}\text{C}$ ,  $^{13}\text{C}$ , 123-I, gold or other dense particles, positron emitters, and the like. These labels may be detected using appropriate imaging techniques such as single photon emission computed tomography (SPECT), medical resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT), and the like, depending upon the type of label used.

The invention also provides a method of treating dependency on addicting substances. A therapeutic neurologic agent which is a receptor active agent capable of binding to a receptor for an addicting substance such as caffeine, nicotine, or cocaine, cannabinoids such as marihuana, opiates such as heroin, and other narcotics may be administered intranasally according to the method of the invention. Preferably, the agent is capable of altering or blocking the neural receptor such that the action and/or uptake of addicting substances is hindered and/or blocked. Receptor active agents capable of altering and/or blocking neural receptor sites include, for example, naloxone, propiram, nalorphine, cyclazocine, methadone, MET-enkephalin, LEU-enkephalin, beta-endorphin, hexamethonium, mecamlamine, QNB, propranolol, phentolamine, pimozide, chlorpromazine, haloperidol, and reserpine, lithium, and carbamazepine, the latter three agents being capable of activity other than receptor effects. Compositions useful in the treatment of dependency on addicting substances preferably comprise an amount of a receptor active agent effective to block receptors for the addicting substance in a pharmaceutically-acceptable liquid carrier. Agents may be further combined with a lipophilic adjuvant. Optionally, odorant substances may be added to the composition. A preferred composition useful in the treatment of, for example, heroin addiction, is the combination of an amount

of naloxone effective to block heroin receptors in the brain, in combination with an appropriate amount of phosphatidylserine to provide lipid vesicles and/or micelles, in a pharmaceutically-acceptable liquid carrier.

5           Another method of treating dependency on addicting substances according to the invention comprises intranasally administering a therapeutic neurologic agent which may be capable of modulating enzyme activity so as to hinder and or block the action and/or uptake of  
10           addicting substances. For example, alpha-methyltyrosine, an inhibitor of tyrosine hydroxylase, may be administered to reduce the effects of amphetamine, or carbamazepine may be administered to reduce the effects of cocaine.

          The invention will be described with reference to  
15           various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

20

#### EXAMPLE 1

#### Formulations of Pharmaceutical Compositions

##### Active Ingredients

25

Group 1. 30 $\mu$ M GM-1 ganglioside (GM-1)

Group 2. 3nM nerve growth factor (NGF)

30

Group 3. 300 $\mu$ M phosphatidylserine (PS)

Group 4. 30 $\mu$ M GM-1  
          3nM NGF

35

Group 5. 30 $\mu$ M GM-1  
          300 $\mu$ M PS

Group 6. 3nM NGF  
          300 $\mu$ M PS

40

Group 7. 30 $\mu$ M GM-1  
          3nM NGF  
          300 $\mu$ M PS

To formulate an aqueous preparation of the pharmaceutical composition, one or more of the following substances and/or carriers may be combined with any one of the aforementioned groups of active ingredients: an odorant such as cetralva, microcrystalline cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, polysorbate 80, benzalkonium chloride, phenylethyl alcohol, and dextrose. The preparation is to be maintained at a pH between 4.5 - 8.5. The concentration of active ingredients may follow the guidelines set forth above, but does not exclude the use of other concentrations or active ingredients.

Alternatively, any one group of the aforementioned active ingredients may be combined with propellants such as trichloromonofluoromethane or dichlorodifluoromethane, and delivered by an aerosol spray or similar application means as a non-aqueous preparation. Oleic acid may be added to the mixture as a lubricant.

#### EXAMPLE 2

##### Formulating Micelles and/or Lipid Vesicles

The compositions of Example 1 may further contain micelles and/or lipid vesicles consisting of GM-1 ganglioside and/or phosphatidylserine (PS). To formulate micelles and/or lipid vesicles, the lipid may be exposed to sonication in the aqueous solution of the pharmaceutical composition. Lipid vesicles and/or micelles formed by this procedure may contain in their interior or associated with their surface, other active ingredients such as NGF and facilitate the delivery of these agents into the brain through the olfactory neural pathway.

#### EXAMPLE 3

##### Preparation of Monoclonal Antibodies GLE-17 and TLE-41

A number of monoclonal antibodies (MABs) were prepared as potential diagnostic agents for neurologic disorders such as Alzheimer's disease. Two of these MABs, GLE-17 and TLE-41, were prepared as follows:

Frontal and temporal cortex material with neuropathologically confirmed Alzheimer's disease was homogenized in three volumes of distilled water. Chloroform and methanol were added to the homogenate to  
5 give a 4:8:3 ratio of chloroform:methanol:water and the mixture was stirred overnight. Centrifugation yielded a soluble fraction or total lipid extract (TLE). A portion of the TLE was diluted further with water to yield a  
10 4:8:5.6 mixture which partitioned into organic and aqueous phases. The aqueous phase contained most of the gangliosides and was referred to as the ganglioside lipid extract (GLE).

The TLE and GLE fractions were dried down separately and redissolved in water. They were then  
15 coated by air drying onto Escherichia coli which had previously been stripped using a series of extractions with acid and acetone. Bacteria coated with either TLE or GLE were then used as immunogens. MABs were prepared in mice and screened against immunogen originally.  
20 Subsequent screening was performed by immunofluorescence against Alzheimer's tangle-bearing neurons and by ELISA against TLE and GLE.

Monoclonal antibodies GLE-17 and TLE-41 are IgM MABs which were found to be reactive with neurofibrillary  
25 tangles of Alzheimer's disease. Accordingly, both MABs are capable of acting as diagnostic agents, being capable of distinguishing Alzheimer's brain tissue from normal or non-demented brain tissue. Both MABs were also reactive with brain sulfatide. TLE-41 was also reactive with  
30 certain phosphoproteins, such as casein, by ELISA.

For further description of methods of coating bacterial membranes with antigens for the preparation of monoclonal antibodies, see Galanos, et al., European Journal of Biochemistry 24: 116-122 (1971), and Young, et  
35 al., Journal of Experimental Medicine 150: 1008-1019 (1979).

## WHAT IS CLAIMED:

1. A pharmaceutical composition for use in administering a neurologic therapeutic agent to the nasal cavity of a mammal for delivery of the agent to the brain of the mammal, comprising: an effective amount of the agent for treating or preventing a brain disease or disorder in combination with a pharmaceutically-acceptable component, the agent or the component having properties that enable the agent to be absorbed through the nasal mucosa and transported by means of the olfactory neural pathway to the brain of the mammal.
2. A composition according to claim 1 wherein the agent is capable of treating or preventing the loss of smell.
3. A composition according to claim 1 wherein the agent is capable of providing antiviral, antibacterial, antineoplastic, antiparasitic, anti-inflammatory, antifungal, stimulant, sedative, hypnotic, analgesic, anticonvulsant, antiemetic, anxiolytic, antidepressant, tranquilizer, cognition enhancer, narcotic antagonist or agonist activity, or any combination of activity thereof.
4. A composition according to claim 1 wherein the agent is a trophic factor.
5. A composition according to claim 1 wherein the agent is capable of acting as a neurotransmitter, neuromodulator, nootropic, hormone, hormone releasing factor, hormone receptor agonist or antagonist, enzyme activator or inhibitor, antioxidant, free radical scavenger, metal chelating agent, or of altering the activity of ion channels.

6. A composition according to claim 1 wherein the component is an appropriate amount of lipophilic micelles or liposomes, odorant agent or any combination thereof.

7. A composition according to claim 6 wherein the agent is contained within the micelles or liposomes.

8. A composition according to claim 6 wherein the micelles or liposomes are capable of transporting the agent through the nasal mucosa to the brain.

9. A composition according to claim 6 wherein the odorant agent is capable of binding to odorant binding protein.

10. A composition according to claim 6 wherein the odorant agent is capable of enhancing transport of the neurological agent to olfactory neural receptors.

11. A composition according to claim 1 further comprising combining the agent with a carrier.

12. A composition according to claim 1 wherein the agent is transported by means of the olfactory neural pathway into the olfactory bulb, olfactory-connected areas of the brain, the hippocampal formation, amygdaloid nuclei, nucleus basalis of Meynert, locus ceruleus, brainstem raphe nuclei, or any combination thereof.

13. A composition according to claim 1 wherein the agent is transported to the brain by means of the olfactory system by transneuronal anterograde transport, transneuronal retrograde transport, or any combination thereof.

14. A composition according to claim 1 wherein the agent is transported to damaged neurons in the brain.

15. A composition according to claim 1 wherein the agent is administered to the nasal cavity as a powder, spray, drops, gel, ointment, injection, or infusion.

16. A composition according to claim 1 wherein the agent is first administered to an eye as drops.

17. A pharmaceutical composition for use in the diagnosis of brain disorders in a mammal, comprising: a labeled diagnostic neurologic agent in combination with a pharmaceutically-acceptable carrier, wherein the composition is capable of effecting absorption of the labeled neurologic agent into the olfactory neural pathway of the mammal.

18. A composition according to claim 17 wherein the diagnostic neurologic agent is capable of detecting substructures or biochemical markers associated with a neurologic or psychiatric illness.

19. A composition according to claim 17 wherein the neurologic agent is a monoclonal antibody, polyclonal antibody, or chemical reagent.

20. A composition according to claim 19 wherein the antibody is selectively reactive with glycolipid, sulfolipid, phospholipid, or phosphoprotein antigens.

21. A composition according to claim 19 wherein the antibody is selectively reactive with sulfatide.

22. A composition according to claim 19 wherein the antibody is labeled by means of a reaction with a second labeled antibody.

23. A composition according to claim 17 wherein the labeling agent is radioactive, enzymatic, fluorescent, or any combination thereof.

24. A composition according to claim 17, further comprising an appropriate amount of lipophilic micelles or liposomes, odorant agent, or any combination thereof.

25. A composition according to claim 24 wherein the agent is contained within the micelles or liposomes.

26. A composition according to claim 24 wherein the micelles or liposomes are capable of transporting the labeled neurologic agent through the nasal mucosa to the brain.

27. A composition according to claim 24 wherein the liposomes or micelles are composed of gangliosides, phospholipids, bile salts, detergent-like adjuvants, or any combination thereof.

28. A composition according to claim 24 wherein the odorant agent is capable of binding to odorant binding protein.

29. A composition according to claim 24 wherein the odorant agent is capable of enhancing transport of the neurologic agent to olfactory neural receptors.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 90/07099

| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup><br>According to International Patent Classification (IPC) or to both National Classification and IPC<br>IPC <sup>5</sup> :     A 61 K 9/08, A 61 K 9/72  |   |                                     |   |   |   |   |  |                      |   |     |       |   |   |             |
|--|---|-------------------------------------|---|---|---|---|--|----------------------|---|-----|-------|---|---|-------------|
| <b>II. FIELDS SEARCHED</b><br><div style="text-align: center;">Minimum Documentation Searched <sup>7</sup></div> <table style="width: 100%; border: none;"> <tr> <td style="width: 25%; border: none;">Classification System</td> <td style="border: none;">Classification Symbols</td> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">IPC<sup>5</sup></td> <td style="border: 1px solid black; padding: 5px;">A 61 K</td> </tr> </table> <div style="text-align: center; padding-top: 10px;">           Documentation Searched other than Minimum Documentation<br/>           to the Extent that such Documents are Included in the Fields Searched <sup>8</sup> </div>   |   |                                     | Classification System   | Classification Symbols  | IPC <sup>5</sup>  | A 61 K  |  |                      |   |     |       |   |   |             |
| Classification System  | Classification Symbols  |                                     |   |   |   |   |  |                      |   |     |       |   |   |             |
| IPC <sup>5</sup>   | A 61 K  |                                     |   |   |   |   |  |                      |   |     |       |   |   |             |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category <sup>10</sup></th> <th style="width: 60%; padding: 5px;">Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 30%; padding: 5px;">Relevant to Claim No. <sup>13</sup></th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">           EP, A, 0145209 (FIDIA SPA)<br/>           19 June 1985<br/>           see page 1, lines 5-12; page 3, line 26 - page 4, line 18; page 8, line 23 - page 9, line 6; page 9, line 30 - page 10, line 32; page 11, example 2; page 13, lines 11-20; claims 1,16         </td> <td style="vertical-align: top; padding: 5px;">           1,2,4,5,11,<br/>15,16         </td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="text-align: center; vertical-align: top; padding: 5px;">           ---         </td> <td style="vertical-align: top; padding: 5px;">           24-27         </td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">           Proceedings of the National Academy of Sciences, volume 79, July 1982, Medical Sciences, Plc., T.C. Anand Kumar et al.: "Pharmacokinetics of progesterone after its administration to ovariectomized rhesus monkeys by injection, infusion, or nasal spraying", pages 4185-4189 see the whole document, in particular 4188<br/> <div style="text-align: center; padding-top: 10px;">             ---<br/>             ./           </div> </td> <td style="vertical-align: top; padding: 5px;">           1,2,5,11-16         </td> </tr> </tbody> </table> |   |                                     | Category <sup>10</sup>  | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>            | Relevant to Claim No. <sup>13</sup>   | X   | EP, A, 0145209 (FIDIA SPA)<br>19 June 1985<br>see page 1, lines 5-12; page 3, line 26 - page 4, line 18; page 8, line 23 - page 9, line 6; page 9, line 30 - page 10, line 32; page 11, example 2; page 13, lines 11-20; claims 1,16 | 1,2,4,5,11,<br>15,16 | A | --- | 24-27 | X | Proceedings of the National Academy of Sciences, volume 79, July 1982, Medical Sciences, Plc., T.C. Anand Kumar et al.: "Pharmacokinetics of progesterone after its administration to ovariectomized rhesus monkeys by injection, infusion, or nasal spraying", pages 4185-4189 see the whole document, in particular 4188<br><div style="text-align: center; padding-top: 10px;">             ---<br/>             ./           </div> | 1,2,5,11-16 |
| Category <sup>10</sup>   | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>  | Relevant to Claim No. <sup>13</sup> |   |   |   |   |  |                      |   |     |       |   |   |             |
| X  | EP, A, 0145209 (FIDIA SPA)<br>19 June 1985<br>see page 1, lines 5-12; page 3, line 26 - page 4, line 18; page 8, line 23 - page 9, line 6; page 9, line 30 - page 10, line 32; page 11, example 2; page 13, lines 11-20; claims 1,16  | 1,2,4,5,11,<br>15,16                |   |   |   |   |  |                      |   |     |       |   |   |             |
| A  | ---   | 24-27                               |   |   |   |   |  |                      |   |     |       |   |   |             |
| X  | Proceedings of the National Academy of Sciences, volume 79, July 1982, Medical Sciences, Plc., T.C. Anand Kumar et al.: "Pharmacokinetics of progesterone after its administration to ovariectomized rhesus monkeys by injection, infusion, or nasal spraying", pages 4185-4189 see the whole document, in particular 4188<br><div style="text-align: center; padding-top: 10px;">             ---<br/>             ./           </div> | 1,2,5,11-16                         |   |   |   |   |  |                      |   |     |       |   |   |             |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>14</sup> Special categories of cited documents: <sup>15</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Z" document member of the same patent family</p> </div> </div>   |   |                                     |   |   |   |   |  |                      |   |     |       |   |   |             |
| <b>IV. CERTIFICATION</b> <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none;">           Date of the Actual Completion of the International Search<br/> <div style="text-align: center; padding-top: 10px;">3rd April 1991</div> </td> <td style="width: 50%; border: none;">           Date of Mailing of this International Search Report<br/> <div style="text-align: center; padding-top: 10px;">17.05.91</div> </td> </tr> <tr> <td style="width: 50%; border: none;">           International Searching Authority<br/> <div style="text-align: center; padding-top: 10px;">EUROPEAN PATENT OFFICE</div> </td> <td style="width: 50%; border: none;">           Signature of Authorized Officer<br/> <div style="text-align: center; padding-top: 10px;">F.W. HECK </div> </td> </tr> </table>   |   |                                     | Date of the Actual Completion of the International Search<br><div style="text-align: center; padding-top: 10px;">3rd April 1991</div> | Date of Mailing of this International Search Report<br><div style="text-align: center; padding-top: 10px;">17.05.91</div> | International Searching Authority<br><div style="text-align: center; padding-top: 10px;">EUROPEAN PATENT OFFICE</div> | Signature of Authorized Officer<br><div style="text-align: center; padding-top: 10px;">F.W. HECK </div> |  |                      |   |     |       |   |   |             |
| Date of the Actual Completion of the International Search<br><div style="text-align: center; padding-top: 10px;">3rd April 1991</div>  | Date of Mailing of this International Search Report<br><div style="text-align: center; padding-top: 10px;">17.05.91</div>   |                                     |   |   |   |   |  |                      |   |     |       |   |   |             |
| International Searching Authority<br><div style="text-align: center; padding-top: 10px;">EUROPEAN PATENT OFFICE</div>  | Signature of Authorized Officer<br><div style="text-align: center; padding-top: 10px;">F.W. HECK </div>   |                                     |   |   |   |   |  |                      |   |     |       |   |   |             |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) |  |                       |
|--|--|-----------------------|
| Category *   | Citation of Document, " with indication, where appropriate, of the relevant passages   | Relevant to Claim No. |
| X  | Yie W. Chien et al.: "Nasal systemic drug delivery", 1989, Marcel Dekker, Inc., (New York, US),<br>see pages 18,19, paragraph 1.9; pages 45-48, paragraph 3.3; pages 55-61, table 3.7; pages 82-84; page 293, paragraph 6.4  | 1-3,5,11-18           |
| Y  | ---  | 9,10,19-23, 28,29     |
| P,X  | EP, A, 0351808 (G.D. SEARLE & CO.)<br>24 January 1990<br>see page 2, lines 4-6; page 4, lines 25-27,39-43; page 5, line 53 - page 6, line 20; page 7, formulations F,I; claims 1,8,14,18,21-23   | 1,2,4-8,17, 18,23-27  |
| X  | WO, A, 8604233 (RIKER LABORATORIES, INC.)<br>31 July 1986<br>see page 1, lines 5-9; page 3, lines 1-18; page 4, line 31 - page 5, line 19; page 14,15, example 13; claims 1,2  | 1,2,5-8               |
| Y  | FR, A, 2260329 (FAVIERE R.E.)<br>5 September 1975<br>see the whole document  | 9,10,28,29            |
| Y  | WO, A, 8901343 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA)<br>23 February 1989<br>see page 1, lines 5-10,20-24; page 6, line 30 - page 7, line 19; claims 1,2, 4,9   | 19-23                 |
| P,X  | Journal of the American Pharmaceutical Sciences, volume 79, no.9, September 1990, American Pharmaceutical Association, Plc.,<br>Munir A. Hussain et al.: "Nasal administration of a cognition enhancer provides improved bioavailability but not enhanced brain delivery", pages 771-772<br>see the whole document, in particular page 772 | 1,2,4,5,10-16         |

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9007099

SA 43046

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| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---------------------|----------------------------|---------------------|
| EP-A- 0145209                             | 19-06-85            | AU-B- 569920               | 25-02-88            |
|   |                     | AU-A- 3518984              | 16-05-85            |
|   |                     | BE-A- 900994               | 07-05-85            |
|   |                     | CH-A- 662278               | 30-09-87            |
|   |                     | FR-A, B 2554346            | 10-05-85            |
|   |                     | JP-B- 2011570              | 14-03-90            |
|   |                     | JP-A- 60132920             | 16-07-85            |
|   |                     | LU-A- 85633                | 04-06-85            |
|   |                     | US-A- 4639437              | 27-01-87            |
| EP-A- 0351808                             | 24-01-90            | JP-A- 2124830              | 14-05-90            |
| WO-A- 8604233                             | 31-07-86            | AU-B- 577663               | 29-09-88            |
|   |                     | AU-A- 5306486              | 13-08-86            |
|   |                     | CA-A- 1264297              | 09-01-90            |
|   |                     | EP-A, B 0209547            | 28-01-87            |
|   |                     | JP-T- 62501906             | 30-07-87            |
|   |                     | US-A- 4814161              | 21-03-89            |
| FR-A- 2260329                             | 05-09-75            | None                       |                     |
| WO-A- 8901343                             | 23-02-89            | AU-A- 2387088              | 09-03-89            |

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